ULTRA-SHORT HIGH VOLTAGE PULSES FOR EARLY POSTMORTEM ASSESSMENT OF MEAT QUALITY

U. Pliquett*, M. Altmann** and K. Schoenbach*

*Center for Bioelectrics, Old Dominion University, Norfolk, VA, USA
**Faculty of Agriculture, University of Halle, Germany

upliquet@odu.edu

Abstract: The active electrical behaviour of meat depends on the ATP level of the cells, the density of ATP-ase within the membrane and the integrity of the membranes. After an ultra-short high voltage pulse that permeabilizes the cell membranes asymmetrically, an active potential occurs across a whole cell. Since the cells are in series electrically, this potential is additive, and can be measured between electrodes which are considerably (cm-range) larger than the dimensions of the cells. Since the magnitude of this active electrical reaction relates to the speed of post-mortem metabolism, we suggest it as suitable parameter for predicting the curing process. Our experiments confirm the hypothesis, despite a high variability of the data. Large differences between the active potential before and after high voltage application correspond to a lower drip loss of the meat.

Introduction

If a cell is placed between two electrodes, both membranes facing the electrodes exhibit the same potential difference between the cytosol and the extra-cellular medium. Thus, the voltage measured at the electrodes would be zero. If one side of the cell differs from the other one, e.g. epithelium or a cell permeabilized at only one side, a net voltage appears at the electrode.

Muscle cells are generally expected to be symmetric and thus the active electrical behaviour is not assessable by means of macroscopic electrodes. The voltage across a pair of electrodes with a distance on the order of centimetres would always be zero. A voltage appears only if one electrode penetrates a cell or if the membranes are asymmetrical with respect to the transmembrane voltage. If the cell membranes at both sides facing the electrodes are electroporated [1], the dc-resistance of the tissue decreases, but no change of the voltage is expected. Studying electroporation using a whole cell clamp configuration yields evidence that, due to a superimposed electric field, the hyper-polarisation side develops a higher voltage than the depolarisation side [2]. However, due to the higher resistance at the hyperpolarization side and, therefore, the higher transmembrane voltage, more dramatic structural changes occur within a shorter time at the hyperpolarization side. If a pulse is short enough (ns-range), only the hyperpolarisation side becomes highly conductive, and the transmembrane voltage decreases due to the shunting pathway. If the depolarisation shows only moderate electroporation or is unaffected, its trans-membrane potential remains at the level of the resting potential or slightly below. Since the cytosol is negative with respect to the extra-cellular medium, the sum of the transmembrane potential of all cells between the electrodes results in a voltage with the opposite polarity of the pulse [3].

The electrically created pores reseal after electroporation. Immediately after resealing, only the Donnan-potential exists, due to the fixed negative charges at inner macromolecules. The Na^+/K^+ ATP-ase re-establishes the K^+-gradient which, in conjunction with K^+ - channels, forces the transmembrane potential to return to the resting potential. The overall effect of Na^+-externalization on the trans-membrane potential is small compared to that of the K^+ - pumping. The time and the degree of potential recovery are interesting parameters for the evaluation of the specimen. The potential difference before and after high voltage application is especially important, since this parameter is quickly and easily determined.

Since these parameters depend on the ATP concentration, the density of ATP-ase, as well as the integrity of the membranes, they reflect the actual condition of the cell. Moreover, with this information, it should be possible to predict the time course of post-mortem changes that influence the meat quality, especially the drip loss.

Material and Methods

Animal material 173 pigs of different breeds and ages are used for testing in a commercial slaughterhouse.

The animals are electrically stunned by using 400 V and a current between 1 and 2 A applied across the head and the heart. Eleven centimeters of *Musculus longissimus dorsi* is dissected from carcasses in the slaughter line from between the 8th and the 13th ribs, 30 min post mortem.

The electrical measurements have been carried out in three directions: (1) parallel to the fibres with the anode at the cranial and the cathode at the caudal side

ISSN: 1727-1983

of the sample, (2) vice versa, and (3) with the electrodes perpendicular to the fibres.

The muscle is cut into 3 slices with a thickness of about 3.6 cm. The dorsal and ventral sides are labelled. Two slices were then cut in half. The pieces from one slice were used for electrical measurements parallel to the fibres; the other pieces were used as control. The third slice is used for the measurements perpendicular to the muscle fibres.

The drip loss is determined for each single specimen as well as for the control. To do this, 50 g of fat free tissue is packed in foil for 60 h. Then the fractional water loss is measured.

Apparatus The specimen is clamped between two parallel-plate electrodes (40 x 25 mm) made from stainless steel. Both electrodes are mounted on rails for distance adjustment (1.5 – 5 cm). An inner pair of electrodes is employed for voltage monitoring before, during, and immediately after the high voltage stimulus. Since the active potential after the stimulus is relatively small, it ranges from µV to mV, the construction of these electrodes is crucial. We used silver/silver-chloride electrodes. Silver wire is chloridized in 0.1 M HCl with a current density of 0.1 mA/cm² and placed in a capillary which is filled with 150 mM KCl. A two stroke syringe pump exchanged the KCl within the capillary after each experiment. The end of the 1 mm capillary with a length of 3 cm was pulled to a tip with an opening of 2-3 µm. Both sensing electrodes are 1 cm in length and are mounted on rails for easy insertion into the tissue. The specimen is placed onto the holder and the outer electrodes are attached with a slight pressure to ensure good contact. Depending on the needs of the experiment, the muscle. Then, the sensing electrodes were inserted (Fig. 1). fibers were perpendicular or horizontal to the plane of the electrode

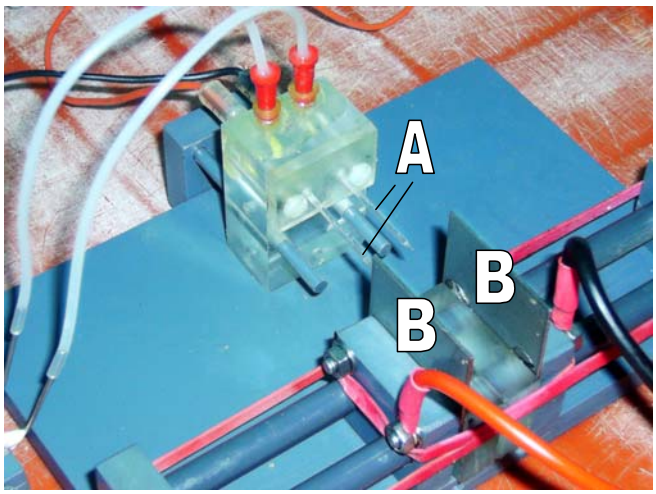

Figure 1: Measuring chamber (A) pipettes with the Ag/AgCl – electrodes (B) outer electrodes

A coaxial cable is charged using high voltage generated by a 20 kV power supply triggered by a spark gap (Fig. 2). The breakdown voltage of the spark gap is set for a value below -20 kV in order to ensure forced breakdown. Immediately after the pulse, the controlling circuit switches the high voltage power supply off, ensuring that only a single pulse is released. The size of the electrodes yields a resistance in the chamber close to 50 Ω when the sample is loaded. Given the cable impedance, the voltage across the electrodes is between 7 and 9 kV, which has been checked under laboratory conditions but not at the slaughterhouse.

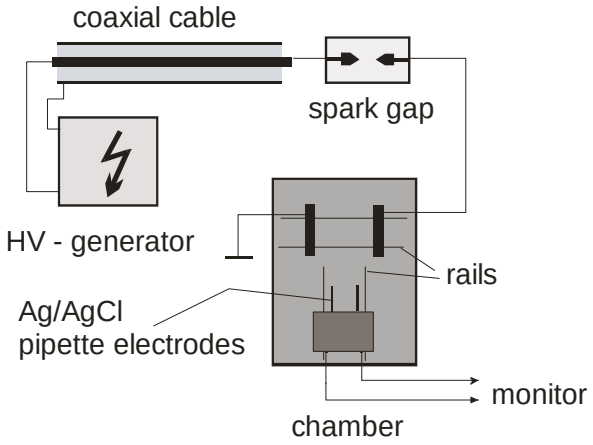

Figure 2: Schematic of the high voltage pulser and the chamber.

The length of the pulse was determined by the length of the coaxial cable. Here we used 24 m, which gave a pulse duration of 240 ns. When a load resistor of 50 Ω was used, the pulse had a nearly rectangular shape. However, we were unable to adjust the resistance of the specimen to match the cable impedance. Therefore, instead of obtaining a rectangular pulse, the pulse was reflected and several declining pulses resulted. For assessing the quality of the stimulus inside the sample, the voltage during the pulse can be monitored. Since it is not feasible to measure the voltage between the inner electrodes directly (electrode resistance > 1MΩ), we used an electro-optical crystal (Pockels Modulator, PM) in order to convert the electric signal into an optical signal (Fig. 3).

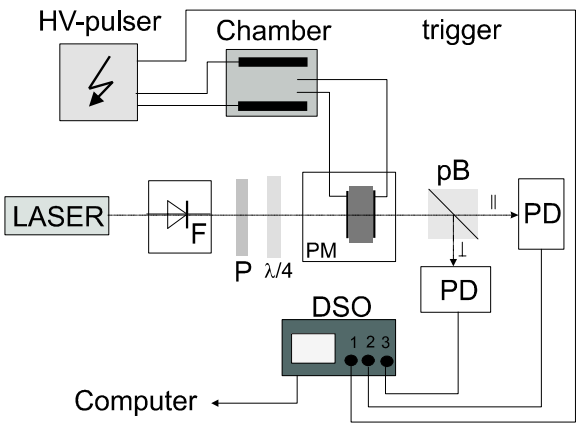

Figure 3: Schematic of the HV-monitor

ISSN: 1727-1983

The LASER (diode pumped YAG–LASER, 532 nm, Gaussian profile, < 1 mW cw) (LOT ORIEL) is circularly polarized using a polarizer (P) and a quarter lambda – plate (λ/4). A FARADAY isolator (Linos Photonics, F) is used in order to prevent reflections into the LASER, thereby destabilizing the output. The POCKELS cell (PM) is a KDP–crystal in a sealed housing (Linos Photonics). For the analysis of the polarisation ellipse, we divided the beam into the polarisation layers using a polarizing beam splitter (PB) and converted the intensity into an electric signal via fast photodiodes (PD, S5872, Hamamatsu, Japan) and 1GHz-operational amplifiers. The bandwidth of the system was limited by the photodiodes at 500 MHz. Both traces, together with a trigger signal obtained from a photodiode, and connected via light guide to the spark (trigger), were monitored using a 500 MHz-4 channel oscilloscope (DSO, Tektronix, TDS520, Beaverton, USA) and transferred to a computer for further processing [4].

µV- voltage monitor for active potential Since the voltage monitor is very sensitive, it was disconnected during the pulse using a high voltage relay. The input stage consists of two FET-input electrometer amplifiers (AD 514, Analog Devices). An instrumentation amplifier yielded the voltage difference between the inner electrodes which was fed into the analog input (12 bit ADC) of a microprocessor (ADµC 812, Analog devices). Additionally, we controlled the high voltage generator as well as the high voltage relay with this microcontroller. The entire device was controlled with a laptop via RS232.

After chloridizing the electrodes, their quality was checked using a 150 mM KCl solution.

Although under lab conditions the device had a sensitivity of 12 µV and the offset was adjusted to zero, in the field we found a significant sensitivity of 93 µV, with an offset of –2.12 mV.

Experimental protocol The optical setup was only used in the lab to validate the equipment. The rest was employed within the slaughter line.

After placing the specimen into the chamber, the voltage across the inner electrodes was monitored for 10 s. Then the high voltage relay connected the HV-generator and a pulse was triggered. 20 ms after the pulse, the relay reset for 1 min, while the voltage is monitored with a sample rate of 2 kS/s. Three more cycles with only 20s of monitoring followed.

Results

The experiments under equal conditions were done in two different slaughter lines at the meat company E.Färber in Torgau and Belgern, Saxony. The company provided excellent facilities for our work in the completely updated plant in Belgern. Fortunately, despite the two different locations, there was no significant variance in the results of our measurements. The measurement procedure was unaffected by the environment.

From each carcass we obtained 3 sets of data, two in the longitudinal and one in the perpendicular direction to the muscle fibres. Since the behaviour of the muscle fibres with longitudinal orientation to the electrodes was surprisingly dependent on the direction (cranial-caudal or caudal-cranial), these sets were treated separately.

Drip loss of the selected samples The carcasses were selected by pH 30 min post mortem to ensure a wide variation in meat quality. A pH value below 6 indicates an eventual high drip loss [7]. Most of the samples (66%) show normal quality (drip loss < 6%) while about 34% is of PSE (pale soft exudative) quality. It should be noted that PSE-quality appears much less frequently in normal production; however, we selected it on purpose to get a large variety in our samples.

Pre-pulse potential If the tissue is homogeneous with respect to the resting potential of the membranes throughout all the cells, the voltage between the inner electrodes is expected to be zero. But if, for instance, one electrode contacts the interior of a cell and the other is within the extra-cellular medium (Fig. 4), a net voltage appears. Here we found a non-zero voltage up to several mV with Gaussian distribution.

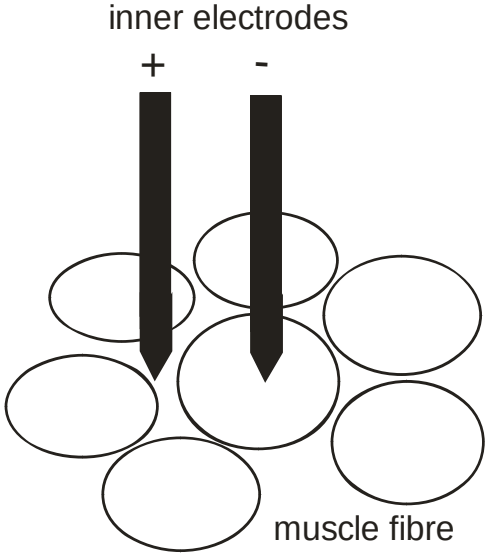

Figure 4: A net voltage appears at the inner electrodes if they perforate cell membranes asymmetrically.

Interestingly, this magnitude of voltage was related to the meat quality as well; low quality, in general, did not show a net voltage above 5 mV. Moreover, this voltage can be measured up to about 1 hour post mortem and disappears with the onset of rigor mortis. Our measurements were done at 30 ± 5 min post mortem, which was the time at which the carcasses arrived at the weighing area.

ISSN: 1727-1983

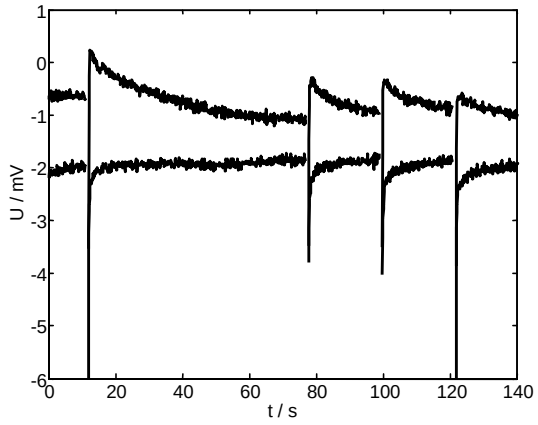

Figure 5: Difference between the voltage at the inner electrodes due to high voltage pulses. The offset before the beginning of the measurement is subtracted. Two examples are shown; low drip loss within 60 h (2.5%, upper trace) and high drip loss (8.4%, lower trace). The electrodes are placed perpendicular to the muscle fibres. The negative pulses (E ≈ 4 kV/cm, t = 240 ns) were applied at 12, 78, 100 and 122 s. Although we had samples with higher and lower drip loss, the data for the figure were chosen for clearly showing the important features.

Voltage difference between before and after the stimulus An example of voltage traces between the inner electrodes is given in Fig. 5. Two samples with different drip loss are shown. Since the shunting of a charged membrane decreases the voltage, it is expected that this is the reason for positive or negative changes in the voltage across the inner electrodes. In this case, one would expect a positive change when the pre-pulse potential difference is negative and vice versa. No active processes need to be involved.

However, as shown in Fig. 6, the pre-pulse voltage U, did not influence the difference in measurements before and after high voltage application, ΔU.

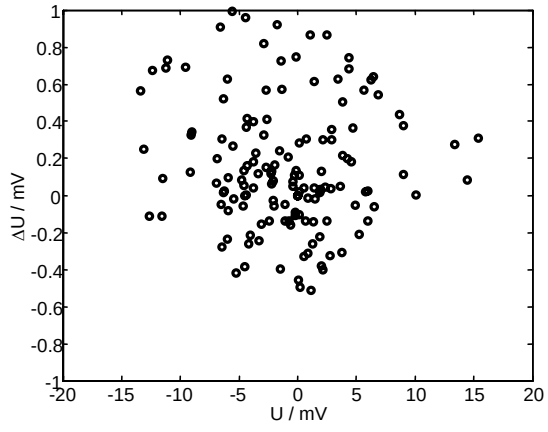

Figure 6: Dot-plot for the difference between the voltage at the inner electrodes after and before a high voltage pulse (ΔU) with respect to the pre-pulse potential (U) for all of the directions of insertion. Only the first pulses are shown. Statistically, later pulses show similar results, but with a smaller difference.

The pre-pulse potential is larger with lower drip loss, i.e. it indicates meat with a higher integrity of the membrane structure. Immediately after a high voltage stimulus, a change in the active potential is found, especially for meat with low drip loss. The difference between the active potential before and after the stimulus is more positive for meat with lower drip loss. (Fig. 7). Measurements taken with the electrodes in a longitudinal orientation showed an obvious correlation to the drip-loss ($r=-0.18$, $P<0.05$ and $r=-0.21$, $P<0.01$); however, when oriented in a perpendicular manner to the fibres, the correlation failed ($r=-0.12$, $P>0.05$). Meat with a high drip loss shows almost no difference. After rigor mortis was reached, no potential difference was found.

Electrode response Since electrode polarization can show an active potential, especially after the application of very high pulses, we tested different electrode materials. Metal electrodes, even iridium sputtered titanium electrodes (which have been successfully used in impedance measurements), showed an active potential after the high voltage pulse, larger than from the tissue. Only reversible electrodes, not exposed to any current flow, are useful as monitor electrodes. The current electrode design sometimes showed an offset of up to several mV, but no changes occurred in the potential after high voltage application when cured meat (>24 h post mortem) or 140 mM phosphate buffered saline was used.

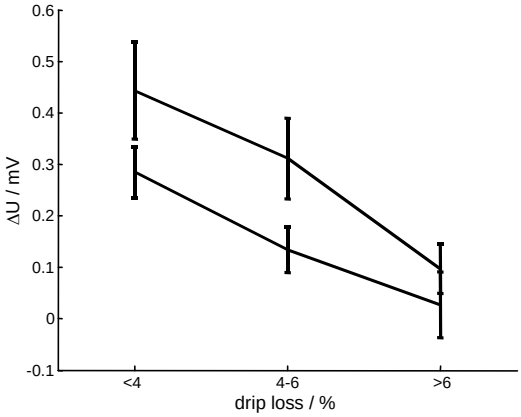

Figure 7: Difference between the voltage before and after the high voltage stimulus for the electrodes longitudinally to the fibres (upper curve: anode cranial; lower curve: anode caudal). The error bars denote the standard error of mean.

Contraction of the muscle due to the high voltage pulse A twitching of the muscle coincides quite well with the appearance of an active potential between the inner electrodes. While the experiments were usually done before the achievement of rigor mortis, it is possible that in a situation with fast post-mortem glycolysis, a considerable calcium level would already exist within the myoplasma, which would fix the

ISSN: 1727-1983

actin/myosin complex and thereby yielding an early rigor. Therefore, electrically induced calcium release from the sarcoplasma cannot cause a contraction of these fibres. The muscles with a low drip loss were even able to relax, a sign that the Ca^{2+} pumps of the sarcoplasmatic reticulum are still active.

Discussion

The appearance of a voltage between the inner electrodes is due to the random placement of the electrodes with respect to the cell membranes. Besides the localization of one electrode within the cytolsol, it can also be placed within the sarcoplasma or within the extra cellular space. The ideal case is one in which both electrodes are outside of muscle cells. Because the plasma membrane of muscle cells is not segmented, as in epithelial cells, no net voltage appears at the inner electrodes (Fig. 8).

In this configuration, no information about the transmembrane potential can be obtained. Once a high electric field is applied, the conductivity of the cell membranes increases by orders of magnitude. If the slew rate of the field is very fast (>10^{10} V/cm·s), and the membrane capacitance is not affected by the field, charging of both the hyperpolarization and the depolarization sides does not show great differences. Also, both sides show an increase in membrane conductance.

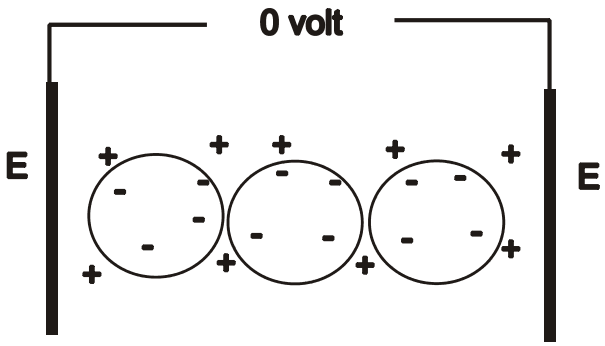

Figure 8: The voltage across electrodes which are by orders of magnitudes larger than the muscle cells should be zero.

However, the time course for affecting the cell membrane is different. This yields a larger effect at the hyperpolarization side, i.e. the anodic side. Using potential-sensitive fluorescent dyes, it has been shown that the transmembrane potential breaks down at the hyperpolarization side to very small values [5].

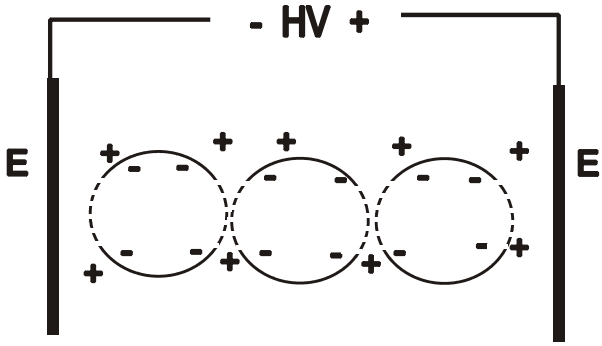

Figure 9: Dramatic increase of the membrane conductance during a high voltage pulse.

Studies of single cells first revealed a great increase for the permeability of small ions at the depolarization side while the resistance at the hyperpolarization side is almost unaffected [6].

This means that for the configuration in Fig. 9, the hyperpolarization side develops a higher transmembrane voltage. Moreover, it is known that a dramatic increase in conductance, interpreted as creation of large pores, happens at the hyperpolarization side, because the transmembrane voltage reached there is higher than at the depolarization side.

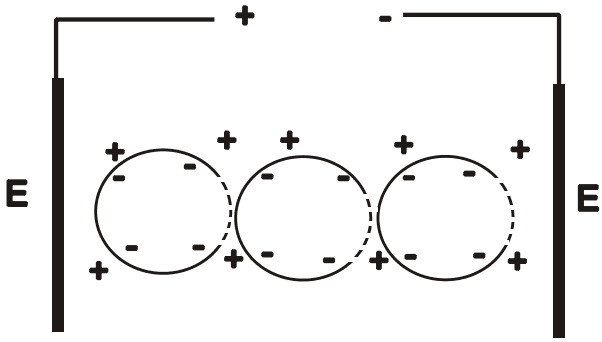

Figure 10: Asymmetric membrane behavior after electroporation

Moreover, it seems likely that in this high electric field the membrane undergoes structural changes, for instance, from a bilayer to a micellar structure. Since these micelles are negatively charged, they are driven away from the cell while, if this also happens at the depolarization side, the micelles are driven into the cell. This means that the recovery at the depolarization side (cathodic side) is much more likely and occurs more readily than the recovery of the membrane at the hyperpolarization side (Fig. 10).

ISSN: 1727-1983

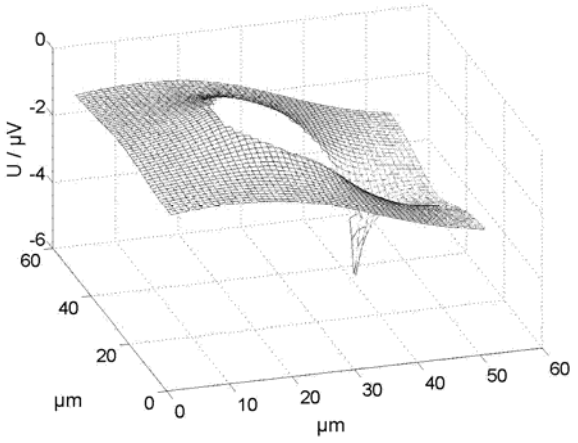

Figure 11: Modeling of voltage in the vicinity of a single-side electroporated cell. (φ_{rest} = -60 mV, $\rho_{membrane}$ = 200 Ωcm^2. $\rho_{extracellular}$ = 100 Ωcm, $N_{pore} = 10^4$, r_{pore} = 0.5 nm)

This yields asymmetric membranes within the muscle. While at the depolarization side the transmembrane potential difference is reestablished to some extent within about 50 ms, at the hyperpolarization side, almost no recovery takes place within this time. Therefore, the potentials from the hyperpolarization sides between the electrodes combine, yielding a net voltage. Since the cell interior is negatively charged with respect to the outer medium, this voltage opposes the pulse polarity. However, since there are shunting pathways around the cell, the voltage difference across a single cell is expected to be very small. A simple simulation (Fig. 11) shows a voltage across a single-side electroporated cell on the order of 6 µV, which adds to 600 µV for 100 cells in series, as expected for the electrode geometry used here.

The re-establishment of the transmembrane potential is closely connected to the membrane integrity, but also to the action of Na^{+}/K^{+} pumps and the functionality of the K^{+}-channels. Moreover, the ability of the membrane to transport K^{+} outside the cell depends on the ATP level. If the ATP disappears due to fast glycolysis, as in the case of PSE-meat, the transmembrane voltage decreases. Since glycolysis yields a decrease in pH, one would expect higher voltages at high pH, which has been reported elsewhere [3].

If the potential change due to a high voltage pulse depends on the metabolism of the cell, which in turn determines the curing kinetics, the active electrical behavior is suitable for predicting the curing process, and finally, the meat quality. In this sense it may be used to determine a quality parameter immediately after killing or at least at the carcass classification stage.

Conclusion

Cells respond to brief and high electric fields, not only with a change in membrane integrity, but also with asymmetric changes of the transmembrane potential. Because of this asymmetry, the active electrical properties can be measured using macroscopic electrodes up to 40 min post mortem. Since the measured potential depends on the physiological state of the cell, which also determines the kinetics and extent of post mortem metabolism, it is closely connected to meat quality.

Acknowledgements

This work was supported by the state of Saxony-Anhalt and partially by AFOSR through a MURI grant: Subcellular responses to narrowband and wideband radiofrequency radiation, administered by Old Dominion University. We thank the meat company E. Färber, Torgau, for their patience and help during our experiments. We thank R. Nuccitelli for helpful discussion.

References

[1] NEUMANN E., SOWERS A., JORDAN C., Electroporation and Electrofusion in Cell Biology, Plenum Press, New York, 1989.

[2] FRANTESCU C.G., WESNER D., PLIQUETT U., NEUMANN E., (2004): Unsymmetrical non-linearity in cell membranes. in: Proceedings of the XII. International Conference on Electrical Bio-Impedance, Gdansk, pp. 41-44.

[3] PLIQUETT U., ALTMANN M., (2004): Active potentials after ultrashort high voltage stimulus. in: Proceedings of the XII. International Conference on Electrical Bio-Impedance Gdansk, pp. 45-48.

[4] PLIQUETT U., ALTMANN M., (2004): Elektrooptischer Effekt zur Spannungsmessung während der Applikation ultrakurzer Hochspannungspulse. in: D. Beckmann, M. Meister (Eds.), Technische Systeme für Biotechnologie und Umwelt, pp. 201-208.

[5] HIBINO M., ITOH H., KINOSITA K., (1992): Time courses of cell electroporation as revealed by submicrosecond imaging of transmembrane potential, Biophys. J., **64**, pp. 1789-1800.

[6] PLIQUETT U., FRANTESCU C.G., NEUMANN E., (2002): Unsymmetrische Elektroporation bei Depolarisation und Hyperpolarisation von CHO-Zellmembranen. in: D. Beckmann, M. Meister (Eds.), Technische Systeme fur Biotechnologie und Umwelt, Institut fur Bioprozess- und Analysenmesstechnik, Heilbad Heiligenstadt, pp. 135-142.

[7] HONIKEL K.O., SCHWÄGELE F., (1998): Biochemische Prozesse der Fleischbildung, in: Branscheid (Eds.), Qualität von Fleisch und Fleischwaren, Frankfurt/Main, pp. 593-613

ISSN: 1727-1983